

Novel *ABCA1* compound variant associated with HDL cholesterol deficiency

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Abstract

The recent discovery of an ATP-binding cassette transporter, *ABCA1*, as an important regulator of high density lipoprotein (HDL) metabolism and reverse cholesterol transport has facilitated the identification of novel variants associated with HDL cholesterol deficiency states. We identified a subject with HDL cholesterol deficiency (4 mg/dl) who developed and died of complications related to cerebral amyloid angiopathy (CAA). The proband had a compound heterozygous mutation. One mutation was a G3295T substitution with conversion of asparagine to tyrosine (D1099Y) in *ABCA1*. The single-base substitution at codon 1099 resulted in the abolition of an *RsaI* cleavage site. The proband and affected individuals having another mutation were heterozygotes for T5966C with phenylalanine converted to serine (F2009S). The presence of the T5966C mutation was detected by restriction digestion with *HinfI*. These variants were not identified in over 400 chromosomes of healthy subjects. In the kindred, family members heterozygous for the *ABCA1* variant exhibited low levels of HDL cholesterol. Direct sequencing of all coding regions and splice site junctions of other HDL candidate genes revealed no additional mutations, indicating that combined defective *ABCA1* alleles may result in familial HDL deficiency. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *ABCA1*; HDL cholesterol deficiency; Mutation

1. Introduction

The ATP-binding cassette transporter, *ABCA1*, is a member of the ABC superfamily of proteins involved in the active transport of substrates across cellular membranes. Like other ABCA subclass transporters, the *ABCA1* protein is characterized by four domains [1–3]; two nucleotide binding domains (NBDs) with conserved Walker A and B motifs are located at the cytosolic face of the membrane. NBDs provide energy for solute transport by hydrolyzing ATP. Another two transmembrane domains consist of six membrane-spanning helices for each, determining the specificity for the transported molecules. *ABCA1* regulates apolipoprotein-mediated lipid removal pathway from cells [4], the initial step of reverse cholesterol transport. Decreased cellular cholesterol efflux by mutant *ABCA1* leads to reduced apolipoprotein (apo)A-I stability and rapid catabolism resulting in high density lipoprotein (HDL) deficiency [4–7].

Several of the ABC transporters have attracted considerable interest due to implication in the pathogenesis of diseases such as cystic fibrosis [8], adrenoleukodystrophy [9], and macular degeneration [10]. Disorder linked to *ABCA1* mutations, Tangier disease (TD) [5,11,12] and familial hypoalphalipoproteinemia (FHA) [13,14], are associated with HDL cholesterol levels of <10 and 20–35 mg/dl, respectively. Both FHA and TD are assigned to the same chromosomal region (9q31).

Observational studies have previously demonstrated an inverse relationship between HDL cholesterol and CAD even when total cholesterol levels are desirable [15]. Because low HDL syndromes are genotypically heterogeneous, understanding their molecular basis could explain the essential role of HDL in plasma cholesterol homeostasis and vascular disease. In view of the importance of *ABCA1* gene mutants in the development of TD, where there is typically virtual absence of HDL cholesterol, we investigated a kindred with similarly low HDL cholesterol but without a history of TD. Our data indicate that a heterozygote compound for mutation in *ABCA1* may cause HDL deficiency without the phenotypic abnormalities associated with TD.

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2. Materials and methods

2.1. Study subjects

The proband, a resident of Overland Park, KS, USA, had markedly reduced HDL cholesterol (4 mg/dl) and apoA-I (8 mg/dl) (Fig. 1, arrow). There was no significant medical history until he suffered a stroke at age 67 and died one year later from complications resulting from cerebral amyloid angiopathy (CAA), as confirmed on necropsy. In addition to the proband, 10 family members of the kindred were screened. There was no history of cardiovascular disease (CVD), and no other CVD risk factors, except for low HDL cholesterol. Moreover, there were no clinical descriptors suggestive of TD. Lipid, lipoprotein and apolipoprotein levels of the proband (shown in bold) and family members of the kindred are shown in Table 1. All subjects gave their informed consent prior to participation.

2.2. PCR amplification and single strand conformation polymorphism (SSCP) analysis

All exons of *ABCA1* were amplified by PCR from genomic DNA using primers and reaction conditions described previously [5,13,14,16] except for the oligonucleotide primers made by us (Table 2). The primers for PCR were made

Table 1

Levels of plasma lipids, lipoproteins and apolipoproteins of the kindred

Subjects	Sex	Age (year)	TC	TG	HDL-C	LDL-C	ApoA-I	ApoB
I-1	M	69	184	130	4	154	8	168
I-2	F	64	279	107	58	200	169	162
II-1	M	39	213	84	51	145	127	103
II-2	F	39	141	74	38	88	111	79
II-3	F	37	159	156	17	111	65	130
II-4	M	35	157	94	30	109	100	106
II-5	M	33	169	80	35	117	108	100
II-6	F	31	178	194	37	102	107	96
II-7	F	23	136	84	27	92	94	92
III-1	F	9	218	106	43	154	112	122
III-2	F	5	212	83	59	136	133	83

TC = total cholesterol; TG = triglyceride; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; Apo = apolipoprotein.

Lipid, lipoprotein and apolipoprotein levels are expressed as mg/dl. Proband is shown in bold letter.

to amplify all coding regions and splice site junctions. Exons of *ABCA1* were designated by the nomenclature of Santamarina-Fojo et al. [17]. For mutation analysis of *ABCA1*, PCR product was mixed with 6X loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF), denatured for 10 min at 96 °C, and placed on ice. This denatured PCR product was electro-

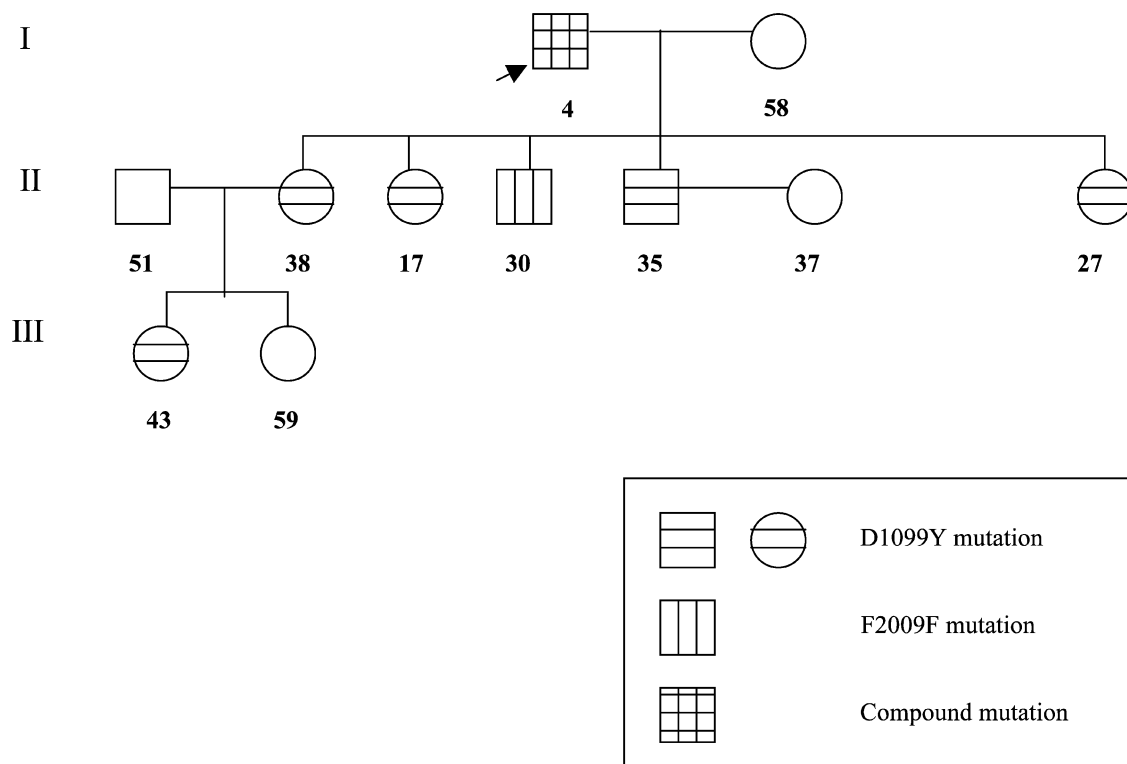


Fig. 1. Pedigree of the kindred showing HDL segregation of D1099Y and F2009S mutations. Horizontal line symbols indicates heterozygote carrier status for the D1099Y mutation. The heterozygous subjects for F2009S mutation are indicated by vertical line symbols. The proband (compound mutation) depicted by horizontal-vertical mixed line symbol is indicated by an arrow. Numbers represent HDL cholesterol levels of the family members.

Table 2
Positions of *ABCA1* exons and sequences of oligonucleotide primers

Exon	Sequence	Annealing Tm (°C)
5	5' -CACTTGGCAGTCACTTCTGT-3' 5' -ACGGATGCAGAGAAGGTT-3'	56
6	5' -TCCTGATATGGCGATGCTCC-3' 5' -TGAGGAAGCTGGAGGCATCA-3'	56
9	5' -CGCCAGCTGTTCAGCATGAG-3' 5' -CATCTTCCTCAGTGCCATTG-3'	55
12	5' -GGATGGCTTAGATTGGACAG-3' 5' -CATGAAGCGAGATATGGTCC-3'	55
17	5' -CAGAGCCTGCTGTCTCTGT-3' 5' -AGACAGCCTCAATGTACCAG-3'	55
21	5' -CAGGCTGACTGTCCAAGA-3' 5' -CCTGACAGCTGGCTTGT-3'	55
23	5' -GGCCGACCAATTATTCTCTCT-3' 5' -CAGCTCACCTTTTTCAGGTA-3'	55
25	5' -TGTCTCTGCTATCTCCAACC-3' 5' -GTCGTCTCTGAGATGCCATA-3'	55
27	5' -TCCTTGTGCCTTCAGATGGT-3' 5' -GGATCAGCAGCATCATCTTC-3'	55
28	5' -TGGCTTCTTGCAAGATCC-3' 5' -GACTCCGTCTGGCAATTA-3'	54
29	5' -AGATTGTCTTGCCAGCTGTG-3' 5' -CTGTTCTGTTGATACCCAGG-3'	55
36	5' -CTGGCATGCAATCAGCTCTT-3' 5' -ACCTCTGAGAGCTGCTGCTT-3'	55
37	5' -TTCTCTCCAGGATGACCACA-3' 5' -AGAGAGCCAGTAGATGACAG-3'	55
39	5' -TTGTGTCTCAACAGGTGGTC-3' 5' -ACATTGTCGGTGAACAGCTC-3'	55

Exons of the *ABCA1* gene are designated by the nomenclature of Santamarina-Fojo et al. [17].

phoresed for SSCP on nondenaturing 8% or 10% polyacrylamide gels at 5–10 W for approximately 24 h at room temperature.

2.3. Sequencing of PCR-amplified DNA

PCR products showing SSCP-shifts of *ABCA1* were isolated by using the Qiagen PCR purification kit and sequenced manually. To identify variants of other primary HDL candidate genes in the proband, all exon regions of apoA-I, lecithin cholesterol acyl transferase (*LCAT*), lipoprotein lipase (*LPL*), phospholipid transfer protein (*PLTP*) and scavenger receptor class B type I (*SR-BI*) genes were amplified and sequenced. The PCR primers were also used for sequencing.

2.4. Restriction enzyme analysis

Genotypic was performed by PCR amplification of exon 23 followed by restriction digestion with *RsaI* for the 3295T mutation, and amplification of exon 45 followed by digestion with *HinfI* for the T5966C mutation. The restriction fragments of exon 23 and exon 45 were separated by 2% agarose gel and 10% polyacrylamide gel electrophoresis, respectively.

2.5. Determination of plasma lipid levels

Plasma samples were obtained in EDTA tubes from individuals who had been fasting for 12–16 h. Levels of plasma total cholesterol and triglycerides were measured by enzymatic colorimetry methods using commercial kits (Boehringer-Mannheim, Indianapolis, IN) and a Hitachi 747 automatic chemistry analyzer. HDL cholesterol levels were determined after precipitation of apoB lipoproteins with heparin sulfate (1.3 g/l) and manganese chloride (0.092 M). ApoA-I and apoB levels were measured by using commercially available immunodiffusion plates (apoA-I, Tago, Burlingame, CA; apoB, Behring Diagnostics, La Jolla, CA). LDL cholesterol levels were calculated by using the formula of Friedewald et al. [18].

2.6. Statistical analysis

Student's *t*-test was used to compare the mean differences in lipid, lipoprotein, and apolipoprotein levels in the presence or absence of *ABCA1* mutation. The designated level of significance was $P < 0.05$.

3. Results and discussion

The first mutation was heterozygous for a G3295T substitution that changes asparagine to tyrosine (D1099Y). The single-base substitution at codon 1099 causes the abolition of a *RsaI* cleavage site. The *RsaI* digested patterns for exon 23 PCR products of human *ABCA1* gene are shown in Fig. 2. The proband and affected individuals having another mutation were heterozygotes for T5966C with phenylalanine converted to serine (F2009S). The presence of the T5966C mutation was detected by restriction digestion with *HinfI* for exon 45 PCR products (Fig. 3). The proband had a compound mutation for D1099Y and F2009S (Fig. 1, arrow). These point mutations were not detected following the screening of more than 400 chromosomes of healthy Caucasians.

Mean lipid levels was compared between carriers (+) and noncarriers (–) of the *ABCA1* variants. Significant differences between (+) and (–) subjects of D1099Y mutation were noted for HDL cholesterol [(+), 27.3 ± 14.6 vs. (–), 47.0 ± 12.9 mg/dl; $P < 0.001$] and apoA-I [(+), 83.0 ± 40.8 vs. (–), 125.4 ± 23.7 mg/dl; $P < 0.001$] levels. The F2009S mutation was also associated with low HDL cholesterol [(+), 17.0 ± 18.4 vs. (–), 40.6 ± 13.9 mg/dl; $P < 0.05$] and apoA-I [(+), 54.0 ± 65.1 vs. (–), 114.0 ± 28.4 mg/dl; $P < 0.05$] levels. Affected family members with the *ABCA1* mutant exhibited low levels of HDL cholesterol, although extremely low HDL cholesterol and apoA-I levels were only detected in the proband having a compound heterozygous mutation.

The amino acids at the D1099Y and F2009S mutation sites are conserved between human and mouse, indicating a potentially important biological function. The D1099Y

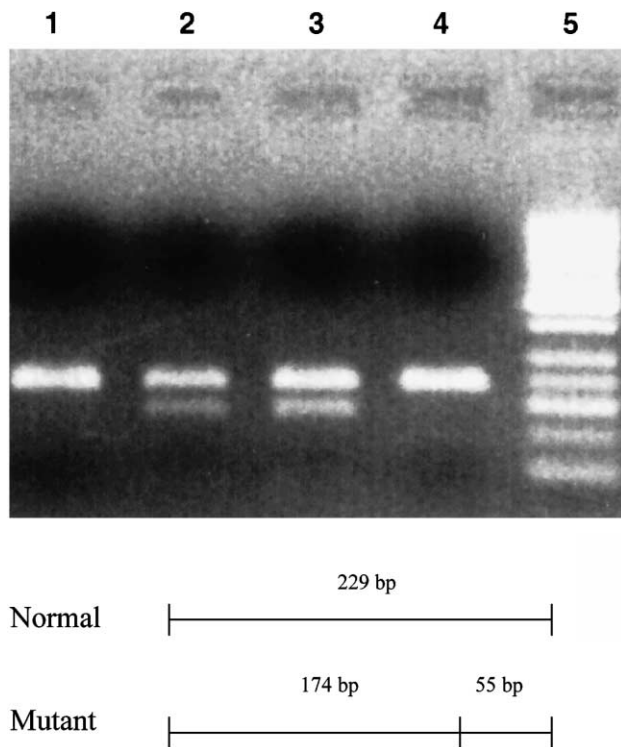


Fig. 2. The *RsaI* digested patterns for exon 23 PCR products of human *ABCA1* gene. In normal subjects, 229-bp fragment corresponding to the full-length PCR amplification product is observed (lanes 1 and 4). Heterozygote for mutation is cleaved to fragments of 229, 174, and 55 bp representing one mutant allele and one normal allele (lanes 2 and 3). Lane 5 is 50-bp DNA ladder marker (MBI Fermentas). The sizes of *RsaI*-digested band in mutant and normal PCR products are depicted at the bottom.

mutation occurs in a highly conserved hydrophobic linker region between the sixth and seventh helix of each transmembrane domain of the ABCA1 protein, suggesting that it may be more likely to impair the specificity for the transported molecules. On the other hand, the region affected by F2009S mutation is within the C-terminal NBD. Thus, a mutation in this domain may impair the ATP hydrolysis necessary for transporter activity.

To rule out variants in other primary HDL candidate genes in the kindred, we sequenced all coding regions and splice site junctions for *apoA-I*, *LCAT*, *LPL*, *PLTP* and *SR-BI* genes. However, no additional mutations were identified, indicating that double defective *ABCA1* alleles may result in familial HDL deficiency.

The proband died of CAA associated with very low HDL level at age 68. Isolated low HDL cholesterol was observed at least 5 years before the diagnosis of CAA was established. The very low HDL might be caused by a compound mutation in *ABCA1*, thereby impairing the ATP hydrolysis necessary for transporter activity and the specificity for the transported molecules of ABCA1 protein. While five additional members of this kindred are heterozygous for the D1099Y allele, they are relatively or considerably younger and have not yet manifested CVD. One carrier member for

F2009Y heterozygous mutation also had no cardiovascular symptom and is younger. It is conceivable that subjects with the heterozygous mutation have subnormal or normal HDL levels during youth because age-related decreases in HDL cholesterol and increases in atherosclerosis have coincided with *ABCA1* variants [19]. A longitudinal study in this family may provide further insight as to the extent of the association between the *ABCA1* variant identified herein and CVD.

In conclusion, the novel *ABCA1* mutation identified in this study segregated with low HDL cholesterol in the kindred, and was not detected in more than 400 chromosomes of healthy subjects. The proband, a compound heterozygote for the two variants, neither manifested TD nor CVD. Whether mutations in *ABCA1* reported herein contribute to the development or progression of atherosclerosis will need to be explored in further studies. The present study provides additional data demonstrating the complexity, diversity and marked phenotypic heterogeneity of low HDL syndromes.

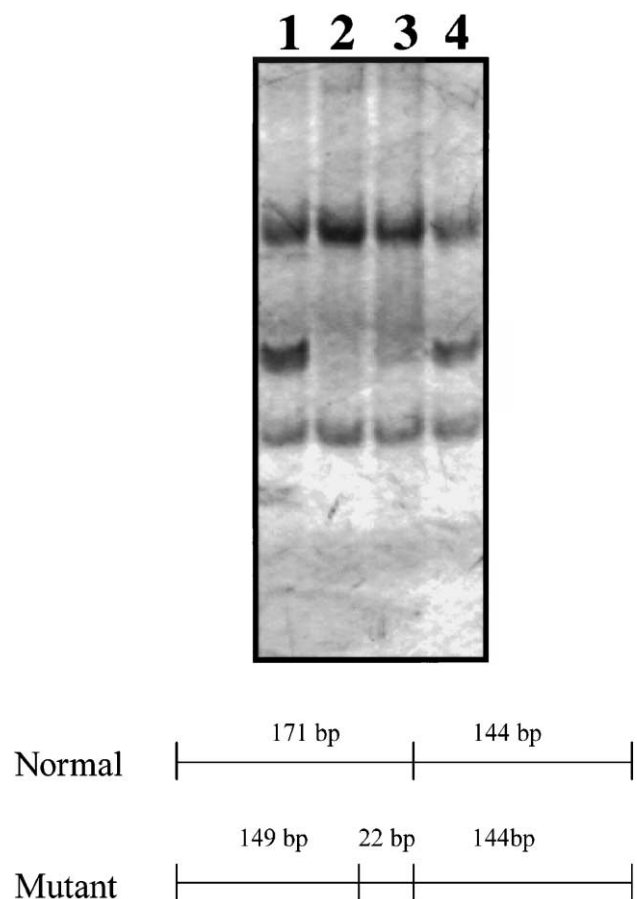


Fig. 3. Restriction digestion analysis of carriers and noncarriers. The presence of the T5966C mutation was detected by restriction digestion with *HinfI*. Heterozygous carriers for the T5966C mutation are shown in lanes 1 and 4. And lanes 2 and 3 are digested patterns of noncarriers. The sizes of *HinfI*-digested band in mutant and normal PCR products are depicted at the bottom.

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